Trakia Journal of Sciences, Vol. 4, No.1, pp 27-32, 2006

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Original Contribution

COMPARATIVE STUDY OF KARYOTYPE MUTABILITY IN INBRED AND OUTBRED RABBITS AFTER GAMMA IRRADIATION

S. Tanchev*, S. Georgieva, E. Zhelyazkov, B. Popov

Department of Genetics, Breeding and Reproduction; Faculty of Agriculture
*Department of Medical Genetics, Faculty of Medicine,
Trakia University, 6000 Stara Zagora, Bulgaria

ABSTRACT

The purpose of the present study was to perform a comparative analysis of karyotype mutability in inbred and outbred rabbits after gamma irradiation. Twelve adult Female rabbits were studied. The rabbits were divided according to their breed, level of inbreeding and genotype features. They all received whole-body irradiation at dose density of 24 cGy/min. 100 Metaphase plates from each rabbit were analysed and the observed structural and ploid chromosome alterations described. Results showed that gamma irradiation at the dose used produced significant increase in chromosome aberrations in inbred animals than in the outbred ones. There was higher percentage of dicentric chromosomes and polyploidy in the inbred. After irradiation heterogeneous rabbits exhibited higher chromosome stability than the homogenous groups.

Key words: radiosensitivity, inbreeding, chromosome aberrations, gamma irradiation

INTRODUCTION

Ionised radiation (including X rays and gamma rays) provokes mutations and cell transformations inducing single or double destruction of DNA molecules and consequently, chromosome instability and carcinogenesis (1, 2) From cytogenetic methods used for evaluation of radiation effect the metaphase test for detection of induced chromosome aberrations could be used for assessment of radiosensitivity in animals and humans (3).

The various organisms and the different cellular systems do not react uniformly to the same parameters of irradiation. Numerous factors modulate these reactions and they include the following: epigenetic such as age because of age-related reduction of DNA reparative capacity of cells (4, 5); the physiological state, for instance pregnancy because they alter hormonal status (6, 7) as well as others, the role of genetically determined radiosensitivity being essential (8). It was found that the genetic environment could influence the frequency and the direction of the effect in radiation-induced mutations.

One method of breeding used in animal husbandry is inbreeding. Its purposeful application in productive animal species or its spontaneous occurrence in various wild animals species could result in significant changes in the genetic structure of the respective population (9, 10).

The principal genetic effect of inbreeding is increase in the homozygosity of offspring. Because of its extensive use in breeding farms, the effect of inbreeding is very well studied with regard to productive and, especially, the reproductive traits of animals (11, 12, 13, 14, 15).

The majority of these studies showed that the narrow inbreeding results in inbreeding depression that is, on its part linked to lower productivity and reduced viability (16).

In nature, all organisms are under the influence of various mutagenic factors, that, depending on its grade, could provoke different chromosome and genetic mutations. Therefore, the capacity and the pattern of

* **Correspondence to:** *Assoc. Prof. Dr Svetlin Tanchev, Trakia university, Rectorate, St. Zagora 6000, E-mail: rector@uni-sz.bg*

reaction of inbred animals to ionised radiations are intriguing. The present study aimed to perform a comparative analysis of karyotype mutability of inbred and outbred rabbits following gamma irradiation.

MATERIAL AND METHODS

Twelve adult female rabbits of age 8 months and live body weight 4.0–4.5 kg were used in the study. The rabbits were reared under uniform conditions from their birth to the beginning of the study. Their distribution by breed, level of inbreeding (determined by the method of Wright) and genotype features were as follows: California rabbits – purebred homogenous – (3 outbred and 3 inbred Fx=0.25); mixed-breed rabbits – heterogeneous crosses – (3 outbred and 3 inbred Fx =0.25).

The division of rabbits into "homogeneous" and "heterogeneous" groups was conditional. Thus, the higher theoretical similarity between genotypes in purebred California rabbits and the expected higher heterogeneity in crosses was expressed.

All rabbits were subjected to whole-body irradiation using a ^{60}Co gamma equipment (Rokus) at dose density of 24 cGy/min. The exposure time was calculated according to the geometrical parameters of the source, the distance and power of the source (17).

Chromosome preparations were procured by short-time cultivation of peripheral blood lymphocytes via a micromethod described by Hungerford (18). From each individual of each group, 100 metaphase plates were analysed and the total number of damaged cells, the number of dicentrics, ring chromosomes and polyploid cells were determined.

The data were statistically processed by SINGLE FACTOR ANOVA using the STATISTICA software.

RESULTS AND DISCUSSION

The data about chromosome changes after the gamma irradiation are presented on **Table 1**. The highest percentage of cells with damaged chromosomes were observed in the group of California inbred rabbits (41.6%), followed by California outbreds. The frequency of abnormal cells in crosses was lower. The comparison of results showed no significant differences between groups with regard to this parameter despite the observed trend towards higher grade of cell damage in purebred (homogenous) types. This tendency was even more visible for chromosome aberrations. For instance, the analysis of chromosome fragments (**Figure 1**) by groups showed again the highest values in California inbred rabbits. Furthermore, the difference between them and all other groups was significant ($p<0.05$). The incidence of fragments in inbred crosses was relatively high (25.3%), but the lowest – in outbred groups. With regard to dicentrics (**Figure 2**) there was not a significant difference among groups except for the fact that their prevalence in purebred homogenous rabbits was slightly higher. The differences were not significant.

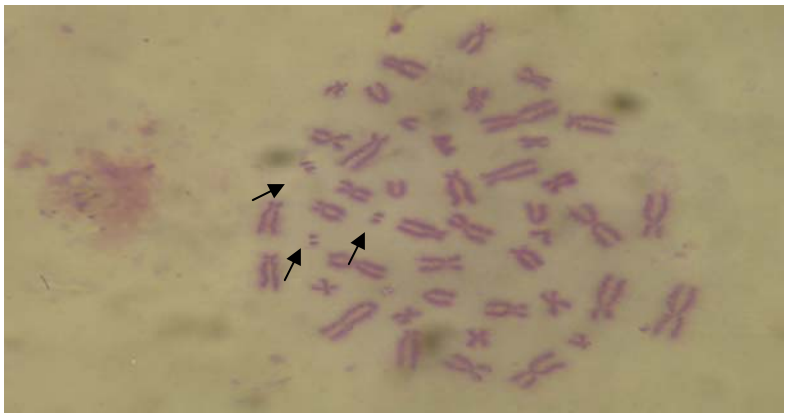

Figure 1. *Fragments*

Table 1. *Chromosome aberrations in rabbits after gamma irradiation (4.0 Gy)*

Group№	***Breed affiliation and genotype features***	***Number of studied metaphase plates***	***Cells with damaged chromosomes***		***Chromosome aberrations***						***Polyploid cells***	
					Fragments		***Dicentrics***		***Rings***			
			number	***%***	***number***	***%***	***number***	***%***	***number***	***%***	***number***	***%***
1	2	3	4	5	6	7	8	9	10	11	12	13
I	California rabbits – outbred	300	118	39,30	181	21,91	109	24,66	8	20,00	8	2,70
II	California rabbits – inbred	300	125	41,67	255	*30,87	125	28,28	15	37,50	13	4,33
III	Outbred crosses	300	111	37,00	181	21,91	101	22,85	7	17,50	5	1,67
IV	Inbred crosses	300	110	36,70	209	25,30	107	24,21	10	25,00	12	4,00
	Total	1200	464	38,66	826		442		40		38	3,16

Note: ****** *significant differences*

The percentages of fragments, dicentric and ring chromosomes are calculated vs the total number of chromosome aberrations of the respective column.

The percentage of cells with damaged chromosomes and the percentage of polyploid cells are calculated vs the number of studied metaphase plates.

More obvious inter-group differences were present in ring chromosomes (**Figure 3**) and polyploid cells (**Figure 4**), where the percentage of the respective chromosome abnormality was higher in inbred rabbits than in the outbred varieties. The differences were nearly significant.

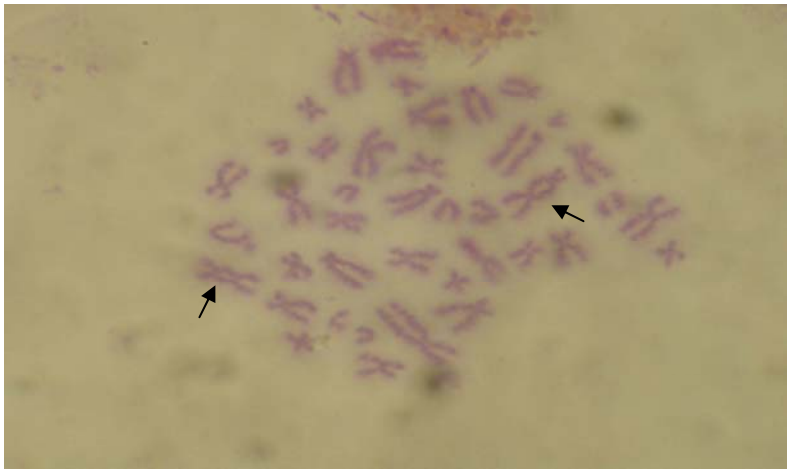

Figure 2. *Dicentrics*

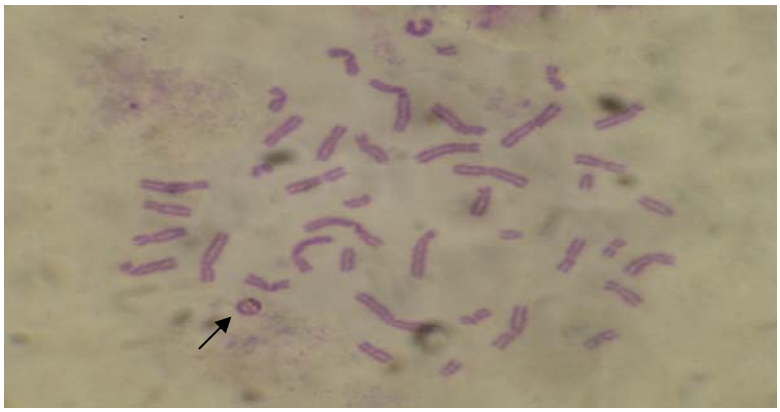

Figure 3. *Ring*

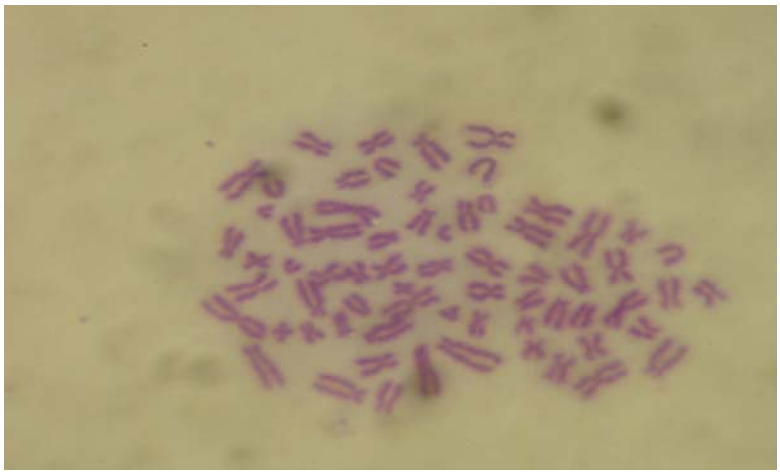

Figure 4. *Polyploidy*

The relative incidence of chromosome aberrations in domestic rabbits, observed by us, was similar to those reported by other authors (19, 20, 21)

The one-way analysis of variance (**Figure 5**) showed that the studied parameters of karyotype damage was significant in each of the breeds ($p<0.05$)

The results of the cytogenetic analysis showed that induced damages were higher in purebred California (homogenous) rabbits than in heterogeneous crosses. An important fact was the finding, that inbred homogeneous and heterogeneous groups showed a higher frequency of chromosome aberrations and, primarily, of the single and double fragments

compared to outbred animals. Our data for karyotype mutability in inbred and outbred animals could hardly be compared, as there were no similar studies in the available literature. The only analogous study in irradiated inbred and outbred mice was that of Touchberry et al. (22). They observed a considerable reduction in the total number and the number of live-born offspring in first and second inbred generations (F_1 and F_2) compared to outbred mice. It is concluded that one of the causes was the induction of a higher percentage of dominant lethal mutations in inbred mice.

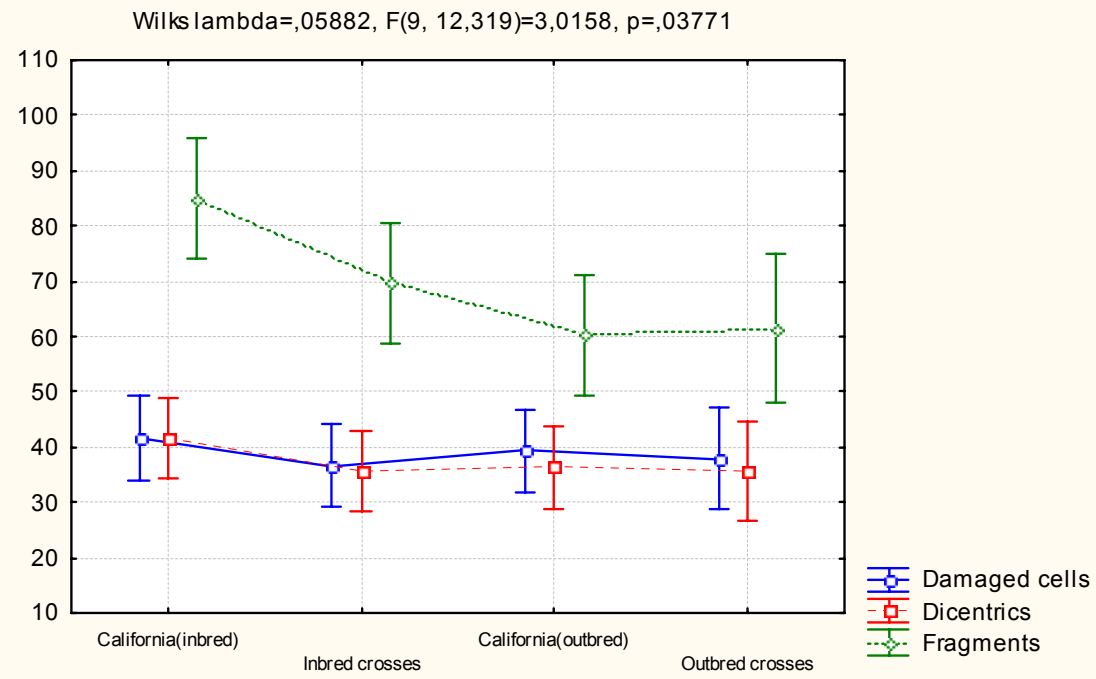

Fig. 5. Number of damaged cells and chromosome aberrations in relation to breed affiliation and genotype features of rabbits, irradiated with gamma rays.

On the other side, it is known that members of inbred populations were characterized by a decreased viability and slower reactions to the effect of various environmental factors unlike outbred populations (23). Most probably, one of the numerous reasons for that is the higher karyotype instability in inbred animals.

The results of the present study enabled us to conclude that the type of breeding – inbreeding, outbreeding or crossing, and the increased homozygosity or heterozygosity, were important for karyotype mutability of the progeny to a certain extent. The rabbits, product of narrow inbreeding (Fx=0.25), exhibited higher karyotype instability than the case with the outbred varieties. The similarity and divergence in genotype of original parents played a definite role in that connection.

REFERENCES

1. Breimer, L.H. Ionizing radiation-induced mutagenesis. *Br. J. Cancer* 57, 6–18 (1988).
2. Morgan, W.F., Day, J.P., Kaplan, M. I., McGhee, E. M. and C. L.Limoli. Genomic instability induced by ionizing radiation. *Radiat .Res.* 146, 247–258 (1996).
3. Sankaranarayanan, K. Ionizing radiation and genetic risks. X. Thepotential "disease phenotypes" of radiation-induced genetic damage in humans: Perspectives from human molecular biology and radiation genetics. *Mutat. Res.* 429, 45–83 (1999).
4. Bolognesi, C., Abbondandolo, A., Barale, R., Casalone, R., Dalpra, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberni, L. and S. Bonassi. Age-related increase of baseline frequencies of sister chromatid exchange, chromosomal aberrations, and micronuclei in human

lymphocytes.*Cancer Epidemiol. Biomarkers Prev.* 6, 249–256 (1997).
5. Wei, Q., Matanoski, G.M., Farmer, E.R., Hedayati, M. and L. Grossman. DNA repair and aging in basal cell carcinoma: A molecular epidemiology study. *Proc. Natl. Acad. Sci. USA* 90, 1614–1618(1993).
6. Ricoul, M., Sabatier, L. and B. Dutrillaux. Increased chromosome radiosensitivity during pregnancy. *Mutat. Res.* 374, 73–78 (1997).
7. O'Brien, S.N., Larcom, L.L. and E.G. Baxlet. Correlates of plasmacortisol and DNA repair in human peripheral lymphocytes: Suppression of repair in women taking estrogen. *Horm. Res.* 39, 241–246 (1993).
8. Hall, E.J., Brenner, D.J., Worgul, B., L. Smilenov. Genetic susceptibility to radiation. Advances in Space Research 35, 249–253. (2005).
9. Ferraz, J.B.S., Johnson, R.K., Van Vleck, LD., Use of animal models to estimate the effects of inbreeding on growth and carcass traits of rabbits. *Journal of Applied Rabbit Research*. 15: 143-157. 1992.
10. Rastogi, R.K. and E. Heyer, Effect of inbreeding on rabbit performance in Trinidad. *Journal of Applied Rabbit Research*. 15: 158-165. 1992.
11. Miros, V.V., Mikhno, V.I. and N.I. Skyarova. Inbreeding in rabbits. *Sel'skokhozyaistvennaia Biologiya*. 6:72-74, 1977.
12. Farghaly, H.M., Effects of inbreeding on doe's performance traits in closed commercial rabbit populations in Egypt. 7th World Rabbit Congress, Valencia. 2000.
13. Rothschild M.A. and A. Ruvinsky. The genetics of the pig.. CAB International. 1998.
14. Ercanbrack, S.K., Knight, A.D. December. Effects of inbreeding on reproduction and wool production of Rambouillet, Targhee, and Colombia ewes. *J. Anim. Sci*. 69 (12): 4734-4744. 1991.
15. Johnson, R. K., Nielsen, M.K. and D.S. Casey**.** Responses in ovulation rate, embryonal survival, and litter traits in swine to 14 generations of selection to increase litter size. *Journal of Animal Science*, Vol 77, Issue 3 541-557, Copyright © 1999 by American Society of Animal Science.
16. Sevinga, M., Vrijenhoek, T., Hesselink, J. W., Barkema, H. W. and A. F. Groen. Effect of inbreeding on the incidence of retained placenta in Friesian horses. *J. Anim Sci*. 82: 982-986. 2004.
17. Kostova, E., Jelev, P., Todorov, B., Determination of conditions for homogenic irradiation of animals, using the gamma source "Rocus". In: Scientific works of the Faculty of Veterinary Medicine. St. Zagora, vol.1. Zemizdat, Sofia (Bg), 1985.
18. Hungerford, D. A., Leucocytes cultured from small innocula of whole blood and preparation of metaphase chromosomes by treatment with KCL. *Speculations in Science and Technology*. 40, 333-335, 1965.
19. Leonard, A., Gebber, G.B., Decat, G., Leonard, E.D., The radiosensitivity of chromosomes of rabbit lymphocytes. *Mutat. Res*. 45, 223-231, 1977.
20. Scott, D., Bigger, T.R.L., The relative radiosensitivities of human, rabbit and rat-kangaroo chromosomes. *Chromosoma*, 49, 185-203, 1974.
21. Bajerska, A., Linieski, J., The yield of chromosome aberration in rabbit lymphocytes after irradiation in vivo and in vitro. *Mutat. Res*. 27, 271-284, 1975.
22. Touchberry, R. W. and F. A. Verley Some effects of X irradiation in successive generations on an inbred and a hybrid population of mice. *Genetics* 50:1187-1209, 1964.
23. Reed, D.H., Lowe, E.H., Briscoe, D.A. and R. Frankham. Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage. *Evolution Int J Org Evolution*. Aug; 57(8):1822-8, 2003.